

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph of the published PCT application beginning at page 2, line 18, with the following amended paragraph:

So far, research has mainly been focused on decreasing proteolytic degradation *in planta*. For example, one strategy to overcome the proteolysis problem in plants is the targeting of proteins to organelles and direct their accumulation in sub-cellular compartments where the protein is more stable. Different studies have demonstrated an increase in intracellular accumulation of a protein of interest, such as antibodies or vicilin when targeted to the endoplasmic reticulum using a carboxy-terminal signal KDEL (**SEQ ID NO:1**) (Tabe et al., 1995, J. Plant Sci. 73:2752-2759). instead of being targeted to the vacuole. However, although this strategy helps prevent proteolysis during expression of recombinant proteins, it does not reduce the risk of proteolysis at the time of extraction. During extraction, plant cells are disrupted and then release various compounds into the medium, including proteases, that may severely alter the integrity of recombinant proteins.

Please replace the paragraph of the published PCT application beginning at page 21, line 13, with the following amended paragraph:

Using the method described in example III, it may be possible to identify which protease activity is responsible for the degradation of a specific clinically useful protein. From there, it would be interesting to be able to find a specific protease inhibitor to selectively abolish the protease activity. The use of synthetic fluorometric protease substrates was investigated towards this application. Fluorimetric protease substrates are useful to determined the potential of various diagnostic or recombinant PIs on the inhibition of specific plant proteases. Leaf proteins were extracted (1:3 w/v) in 50 mM Tris-HCl pH 7.5 containing 10 mM β -mercaptoethanol, and protein content was adjusted to a final concentration of 1 mg/ml with extraction buffer. A master reaction mix was prepared by mixing 1080 μ l extraction buffer, 108 μ l plant extract and 12 μ l of either 1mM Ala-Ala-Phe-MCA, 1 mM suc-Ala-Ala-Pro-Phe-MCA (**SEQ ID NO:2**), 1mM suc-Leu-Val-Tyr-MCA or 1mM Bz-Arg-MCA. One hundred μ l of the master mix were

dispensed in 96-well microplates and 5 μ l of 100 mM PMSF (inhibitor of serine proteases), 1 mM aprotinin (inhibitor of serine proteases), 10 mM chymostatin (inhibitor of serine proteases and some cysteine proteases), 1 mg/ml α -1 antichymotrypsin (inhibitor of chymotrypsin-like proteases), 10 mM leupeptin (inhibitor of trypsin-like proteases and some cysteine proteases), 1 mM pepstatin (inhibitor of aspartate proteases), 100 mM E-64 (inhibitor of cysteine proteases), recombinant CDI (cathepsin-D inhibitor; inhibitor of aspartate proteases), recombinant OCI (oryzacystatin I; inhibitor of cysteine proteases), recombinant CCII (corn cystatin 2; inhibitor of cysteine proteases) and recombinant PMC8 (potato multicystatin domain 8; inhibitor of cysteine proteases) were finally added to the reaction mixture. Fluorescence intensity was measured 100 times over a 5,000-sec period at 30°C using a Fluostar Polastar Galaxy™ fluorimeter (BMG Lab Technologies), with excitation and emission filters of 485 nm and 520 nm, respectively. Protease activity, expressed in units of fluorescence per min., corresponded to the slope of the emission curve. As shown in Figs. 3 and 4, various types of proteases may be considered as possible targets to decrease protease activities from alfalfa and potato leaves, including serine (e.g., PMSF-, aprotinin, chymotrypsin- and chymostatin-sensitive), cysteine (E-64/cystatin-sensitive) and aspartate (pepstatin-sensitive) proteases.

Please replace the paragraph of the published PCT application beginning at page 24, line 4, with the following amended paragraph:

Similarly to Example V, soluble proteins were prepared from potato (cultivar Kennebec) leaves, separated by Mono-Q chromatography, and submitted to gelatin/PAGE (Fig. 6A), as described in Fig 5A. Protease activity was determined for each chromatographic fraction by fluorimetry using a cathepsin D-specific substrate (MOCAc-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(Dnp)-D-Arg-NH₂) (SEQ ID NO:3) at a final concentration of 6 μ M (Fig. 6B). As depicted in Fig 6C, protease activity in the potato leaf protein fraction showing the highest cathepsin D-like activity (fraction # 13) was dramatically altered by the aspartate-type inhibitor tomato cathepsin D inhibitor 'CDI', identifying CDI-sensitive proteases as interesting targets for the development of strategies aimed at protecting protein integrity via the inhibition of the

plant's endogenous proteases. Noteworthy, our data also show that the inhibition of a single protease (or protease group) may be sufficient to protect a significant part of the proteins present in crude extracts, despite the presence of other (insensitive) proteases in the medium.